An Examination of Commercial Kits for the Determination of Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT) in Serum

M. HOLLANDS, M.A.* and J. E. LOGAN, Ph.D., *Ottawa, Ont.*

Four kits for the detection of serum transaminase based on the spectrophotometric method and 15 kits based on the colorimetric procedure were evaluated. Two kits contained faulty reagents in both the SGOT and SGPT packages. Four of the 15 kits gave results which differed significantly from those of the reference method. The precision of the various kit procedures was adequate in each case for the determinations of SGOT and SGPT. The need to evaluate the adequacy of each kit in a routine operation before relying upon the results obtained with it is stressed.

Quatre trousses commercialisées pour le dosage de la transaminase sérique, par la méthode spectrophotométrique, et 15 trousses utilisant la méthode colorimétrique ont été examinées. Deux trousses contenaient des réactifs défectueux dans les deux emballages (GOT et GPT). Quatre des 15 trousses donnaient des résultats qui différaient sensiblement de ceux que donne la méthode de référence. La précision des méthodes préconisées par les diverses trousses était suffisante dans chaque cas pour le dosage de GOT et GPT sériques. Les auteurs attirent l'attention sur la nécessité d'estimer la valeur de chaque trousse au cours d'une opération de routine avant de se fier aux résultats obtenus.

IN the past 10 years, interest in the determination of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) has increased following the demonstration that elevations of these enzymes in serum indicated tissue damage, particularly in heart and liver. This mounting interest has resulted in increasing numbers of requests for these determinations in the clinical laboratory. The commercial houses have been quick to respond to the demand, and as a result a large number of kits for GOT and GPT determinations are available in Canada. In this communication we present an evaluation of some of these kits carried out in our laboratory.

The transaminase enzymes catalyze the transfer of an amino group from an amino acid to an α-ketoacid. In the case of GOT the amino group of aspartic acid is transferred to α-ketoglutaric acid to form glutamic acid and oxaloacetic acid. With GPT, the amino group of alanine is transferred to α-ketoglutaric acid to form glutamic acid and pyruvic acid. Heart and liver have a rich supply of GOT and liver contains large amounts of GPT.

METHODS

The measurement of transaminase levels in serum by the various kits studied is based on one of two principles, with one exception (Transac). Several of the kits utilize the colorimetric procedure of Reitman and Frankel[1] in which the oxaloacetate and/or pyruvate formed in either the GOT or GPT reaction is combined with 2, 4-dinitrophenylhydrazine to yield a brown-coloured hydrazone which is measured at 505 mμ. in the photometer. Other kits utilize the spectrophotometric method of Karmen[2] in which malic acid dehydrogenase is added to convert the oxaloacetic acid formed by GOT to malic acid with the simultaneous oxidation of the coenzyme $NADH_2$ (reduced nicotinamide-adenine dinucleotide) to NAD (nicotinamide-adenine dinucleotide). In the case of GPT, lactic acid dehydrogenase is used and lactic acid is formed simultaneously with NAD. The decrease in absorbance of $NADH_2$ as it is oxidized to NAD is followed at 340 mμ. by the spectrophotometer in each case. In the case of the Transac kit* for GOT measurement, the oxaloacetic acid is reacted directly with a diazonium salt to form a coloured complex instead of depending on the formation of pyruvate from oxaloacetic acid, as in some colorimetric procedures.

In our evaluations, we have compared those kits utilizing the ultraviolet method against the Karmen procedure as reference, and those kits using the colorimetric technique against the Reitman and Frankel method as reference. The Transac kit was evaluated against the method described by Babson *et al.*[3] With all kits, the manufacturer's instructions for their use were strictly observed.

For transaminase determinations serum specimens obtained at the Ottawa Civic Hospital were used. Optical density readings were made using a Beckman DB spectrophotometer. The cuvette compartment of this instrument was fitted with thermospacers to allow temperature control of the compartment within $\pm 0.5°C$. from a thermostatically controlled circulating bath. Careful timing of the readings during the enzyme reaction was observed in all the studies.

Observations were made concerning the stability of the reagents in the kits and the completeness of the instructions. The reproducibility of results using the various kits was assessed by carrying out duplicate determinations.

From the Clinical Laboratories, Laboratory of Hygiene, Department of National Health and Welfare, and the Department of Medical Education and Research, Ottawa Civic Hospital, Ottawa, Ontario.
*Present address: Department of Pharmacology, University of Manitoba, Winnipeg 3, Manitoba.
Reprint requests to: Dr. J. E. Logan, Clinical Laboratories, Laboratory of Hygiene, Ottawa 3, Ontario.

*Warner-Chilcott Co., Toronto, Ontario.

RESULTS

Serum GOT (SGOT) levels on a large number of specimens determined by the Karmen, Reitman and Frankel, and Babson methods are compared in Table I. The latter two methods (colorimetric) yielded values higher than those by the Karmen ultraviolet spectrophotometric method ($P < 0.05$). A comparison of serum GPT (SGPT) levels by the Reitman and Frankel and the Karmen methods showed lower values with the colorimetric procedure ($P < 0.05$).

TABLE I.—DIFFERENCES BETWEEN VALUES OBTAINED BY COLORIMETRIC METHODS (REITMAN AND FRANKEL AND BABSON) AND VALUES BY THE KARMEN SPECTROPHOTOMETRIC METHOD

SGOT mean values				
Babson	*Reitman and Frankel*	*Karmen*	*P value*	*No. sera*
58	—	56	<0.05	168
—	53	52	<0.05	193
		SGPT		
—	36	40	<0.05	87

The results of determinations of SGOT by the Warner-Chilcott transaminase SGO kit and the Worthington Determatube-SGO kit are compared in Table II with those of the Karmen method. It will be noted that the Worthington kit gave significantly lower values than the reference method ($P < 0.001$), whereas the Warner-Chilcott values did not differ statistically ($P > 0.05$).

TABLE II.—DIFFERENCES BETWEEN VALUES OBTAINED BY COMMERCIAL KITS (SPECTROPHOTOMETRIC) AND VALUES BY THE KARMEN METHOD

SGOT mean values				
Warner-Chilcott	*Worthington*	*Karmen*	*P value*	*No. sera*
76	—	78	>0.05	64
—	68	75	<0.001	19
		SGPT		
20	—	20	>0.05	34
—	14	18	>0.05	8

The results obtained on Warner-Chilcott and Worthington SGPT kits are also assessed in relation to those of the Karmen method (Table II). In this case, the mean values by the Warner-Chilcott procedure agree well with the reference method whereas the Worthington test tends to yield lower values. No statistically significant differences were found, however, but in the latter case only eight specimens were assessed.

Ninety-eight sera were screened with the Dade SGOT screening test to identify SGOT activity levels definitely below, within, or definitely above the limits of 40-60 units. The actual values were determined by the Karmen procedure, and the results are shown in Table III. The separation of the specimens into the three categories by the screening test is confirmed by the quantitative determinations.

TABLE III.—COMPARISON OF RESULTS BY THE DADE SCREENING TEST WITH RESULTS BY THE KARMEN METHOD

Range—Karmen units	*SGOT Screening test results*			*No. sera*
	Normal	*Borderline*	*Elevated*	
<40.....	20	1	0	21
40 - 45.....	2	2	0	4
46 - 50.....	0	15	0	15
51 - 55.....	0	3	0	3
56 - 60.....	1	0	2	3
61 - 65.....	0	1	11	12
66 - 70.....	0	0	6	6
71 - 75.....	0	0	1	1
>75.....	0	0	33	33
Total.....				98

Five commercial kits based on the Reitman-Frankel colorimetric method were evaluated against this procedure. The results of this evaluation for SGOT and SGPT determinations are given in Table IV. For SGOT, Dade and Hyland kits yielded values significantly different from the Reitman and Frankel values. In the case of Dade, the means were higher, whereas with Hyland they were lower. For SGPT, the Dade kit gave statistically significant different results. These values were also higher than the reference procedure.

A check of 45 sera using the Transac kit with the Babson procedure as the reference method revealed no difference between the mean values.

The degree of reproducibility obtained with some of the kits for SGOT and SGPT determinations is shown in Table V. Insufficient numbers of duplicate analyses precluded the determination of precision for the remaining kits. Confidence limits are expressed as a percentage of $\pm$ 3 S.D. divided by the mean value. It will be noted that the reproducibility of the SGOT kits is better than that of the SGPT kits.

Two kits were found to contain faulty reagents. The reagents of the Hellige kit for SGOT and SGPT became mouldy after six weeks' storage in the refrigerator. Directions for their use were not included. The Biochemica "Boehringer" test kit distributed by Calbiochem contained incomplete instructions and its sensitivity fell off almost completely in five weeks, even though the reagents had been refrigerated. Other production lots of these two kits were not examined.

In the case of Sigma, Dade and Uni-Tech products, kits purchased 12-15 months previously were compared with more recently purchased ones (two-three months). When a fresh pyruvic acid standard was prepared for the old kits, no significant differences were found in the values obtained with the old and new kits.

TABLE IV.—DIFFERENCES BETWEEN VALUES OBTAINED BY COMMERCIAL KITS (COLORIMETRIC) AND VALUES OBTAINED BY THE REITMAN AND FRANKEL METHOD

SGOT mean values							
Dade	*Harleco*	*Hyland*	*Sigma*	*Uni-Tech*	*Reitman and Frankel*	*P value*	*No. sera*
55	—	—	—	—	51	<0.01	67
—	36	—	—	—	34	>0.5	51
—	—	49	—	—	51	<0.01	36
—	—	—	53	—	52	>0.8	41
—	—	—	—	62	64	>0.1	27
SGPT							
37	—	—	—	—	35	<0.05	77
—	36	—	—	—	35	>0.3	67
—	—	31	—	—	33	>0.05	32
—	—	—	33	—	35	>0.4	77
—	—	—	—	29	30	>0.8	61

Hemolysis was found to increase the SGOT values significantly but did not affect the results for SGPT even when it was extensive.

Package information in the various kits by nine companies, such as handling precautions, stability and operating procedure, was reviewed. Only two companies mention that 2, 4-dinitrophenylhydrazine should be protected from the light and only one company places an expiration date on its product. Four companies use chloroform and one uses sodium azide as preservative while four others

TABLE V.—PRECISION OF METHODS AND KIT PROCEDURES

Name of method or kit	*SGOT* *Mean value ± S.D.*	*Confidence limits* $\left(\frac{\pm 3\ \text{S.D.}}{\text{Mean}} \times 100\right)$ %
Karmen (u/v)	77 ± 2.1 (40)*	8.3
Reitman and Frankel	58 ± 1.5 (40)	7.9
Babson	51 ± 1.1 (40)	6.7
Transac	67 ± 1.9 (40)	8.6
Warner-Chilcott	67 ± 1.4 (14)	6.4
Hyland	56 ± 1.9 (40)	10.4
Sigma	63 ± 2.6 (10)	12.1
	SGPT	
Wroblewski (u/v)	34 ± 1.6 (40)	14.1
Reitman and Frankel	42 ± 1.5 (40)	10.8
Hyland	47 ± 2.4 (40)	15.3
Sigma	24 ± 1.6 (28)	19.4
Harleco	45 ± 2.1 (20)	13.9
Warner-Chilcott	29 ± 0.8 (10)	8.0

*Number of estimations in each case indicated in parentheses.

do not indicate that any preservative is used. Five provide information on the stability of the enzyme in serum and four mention the stability of the final colour produced. The effect of hemolysis is considered by five manufacturers, and six provide some details on the wave-length setting of the photometer. In two cases (Hyland and Transac) the use of a commercial control serum is recommended as the standard of reference for the test. The Hyland kit includes the sodium hydroxide required in the analyses, and the Sigma Company will provide it on request. Spectrophotometric kits were found to be much more expensive than those using the colorimetric method. Table VI shows the upper limit of enzyme activity in the specimen before dilution is necessary.

TABLE VI.—UPPER LIMIT OF ENZYME ACTIVITY MEASURABLE BEFORE DILUTION REQUIRED

Manufacturer	*SGOT*	*SGPT*
Calbiochem	150 units	150
Dade	165	125
Harleco	165	125
Hyland	200	200
Sigma-505	160	125
Uni-Tech	165	125
Warner-Chilcott (Transac)	350	—
Warner-Chilcott transaminase SGO and SGP	300	300

DISCUSSION

Comparison of data obtained by the Reitman and Frankel colorimetric procedure with data obtained using the Karmen spectrophotometric method is not entirely valid. In the former procedure, the amount of product formed as a result of the transamination reaction is measured, whereas in the ultraviolet method the enzyme activity is determined by following the rate of the reaction. However, since the Karmen method is the one most often used as a reference, comparisons with colorimetric methods have been made. Wroblewski[4] reports that, in the normal range for SGOT, the values by the two methods agree favourably but in the elevated range the ultraviolet results are much higher than the colorimetric. In our data for SGOT the values were divided almost equally between normal and abnormal ones. The application of Student's "t" test indicates that the slightly higher results obtained for the Reitman and Frankel method are statistically significant. For SGPT, in which 33 of the 87 values were greater than 30 units, the mean value by the Reitman and Frankel method was significantly lower than that by the Karmen method. The findings of Reitman and Frankel,[1] based on smaller numbers of comparisons, show differences in the abnormal range

of values that are in the same direction as those we have reported above.

There is no apparent explanation for the significantly lower values obtained for SGOT using the Worthington Determatube kit. It should be pointed out that where large numbers of specimens are to be analyzed by this procedure, several vials of α-ketoglutarate must be reconstituted, and this might be a source of error.

The screening results obtained with the Dade SGOT kit agree well with the quantitative data obtained by the Karmen method. Out of 98 specimens tested only one result would be questionable, i.e. a value of 58 units by the Karmen method which was judged to be normal by the screening kit. No normal specimens (<40 units) were judged to be elevated and no elevated specimens (>60 units) were judged to be normal by this test. In a recent paper, Comfort[5] found this kit quite suitable for screening purposes when additional control sera with known SGOT values were utilized.

Values by the Dade and Hyland SGOT kits differed significantly from those of the Reitman and Frankel procedure. Higher results were also observed with the Dade SGPT kit. An examination of the data indicates that the wider discrepancies were present mostly in the comparisons of the elevated values. This would not constitute any serious drawback in the use of these kits.

The precision data of the various kit methods were found to be acceptable and in our hands were generally better for SGOT determinations than for SGPT determinations. Since the normal values for transaminase activity cover a rather broad range in each case, a greater degree of error may be tolerated if one applies the formula proposed by Tonks[6] than in the case of some of the common chemical determinations. None of the values obtained for the confidence limits would exceed the acceptable limits of error on this basis. Our value of ± 8.3% for the Karmen method for SGOT is in agreement with that quoted by Henry.[7]

In choosing between an ultraviolet method and a colorimetric procedure for the determination of transaminases, the ultraviolet method is generally more rapid and is more accurate. It suffers the disadvantage that the enzyme reagents required are more expensive and vary in stability. Also the laboratory must be equipped with an ultraviolet spectrophotometer. In the colorimetric procedures the reagents are relatively stable and readily available but the procedures are usually more time-consuming and less accurate than the ultraviolet methods.

Considerable variation has been shown to exist in the adequacy of instructions which the manufacturers provide with their transaminase kits. These are very important and particularly so in the case of an enzyme reaction where conditions must be carefully controlled to obtain reliable results. It is probably true that commercial kits are most attractive to smaller hospital laboratories where the technician has limited training in the preparation of reagents or has insufficient time to prepare them because of the high volume of work to be completed throughout the day. This places a considerable responsibility on the manufacturer to ensure that results obtained by using his product can be relied upon by the physician making the diagnosis. Even though the reagents may be properly prepared and packaged in the kit, if notes are not adequate and precautions (proper storage, control of temperature and pH, effects of interfering materials, cleanliness of glassware, etc.) are not stressed in relation to the use of the kit, considerable error may be introduced by an operator even though he has followed all the information provided. Such incorrect information coming from the laboratory can, in some instances, result in the wrong diagnosis being made, or the wrong course of therapy being followed. Transaminase determinations may, on occasion, indicate such major disorders as myocardial infarction or liver damage, and incorrect values could lead to serious consequences. A paper from this laboratory stressing the need to evaluate the capabilities and limitations of commercial kits before accepting them for routine use has been published earlier.[8] Continued vigilance on the part of laboratory personnel is required to ensure that only those products whose performance has been adequately tested are used for laboratory analyses.

SUMMARY

Nineteen transaminase kits sold in Canada were evaluated for accuracy, precision, stability of reagents and for adequacy of instructions supplied. Testing of two kits could not be completed because of the instability of the reagents. Four kits yielded values which were not in agreement with those obtained by the reference method. The precision of the five SGOT kits and the four SGPT kits so tested was found to be acceptable for routine clinical laboratory use. Some kits did not contain instructions that were complete enough to ensure that the operator would be aware of those factors which could introduce serious errors into the performance of the test.

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